PATENT COOPERATION TREATY

	From the INTERNATIONAL BUREAU				
PCT	То:				
NOTIFICATION OF ELECTION (PCT Rule 61.2)	United States Patent and Trademark Office (Box PCT) Crystal Plaza 2 Washington, DC 20231 ETATS-UNIS D'AMERIQUE				
Date of mailing: 03 January 1997 (03.01.97)	in its capacity as elected Office				
International application No.: PCT/JP96/01641	Applicant's or agent's file reference: FP-KS-0434				
International filing date: 14 June 1996 (14.06.96)	Priority date: 14 June 1995 (14.06.95)				
Applicant: HATADA, Yuji et al					
1. The designated Office is hereby notified of its election made. X in the demand filed with the International preliminar 21 November	y Examining Authority on: 1996 (21.11.96) national Bureau on:				
The International Bureau of WIPO	Authorized officer:				

J. Zahra

Telephone No.: (41-22) 730.91.11

Facsimile No.: (41-22) 740.14.35

1211 Geneva 20, Switzerland

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference		See Notification of	of Transmittal of International	
FP-KS-0434				
International application No.	International filing date (day/	month/year) Pr	iority date (day month year)	
PCT/JP 96/01641	14/06/1996	1	4/06/1995	
International Patent Classification (IPC) o	r national classification and IPC			
	C12N15/56			
Applicant				
KAO CORPORATION et al.				
heen amended and are the h	sheets, including to ANNEXES, i.e., sheets asis for this report and/or sheets 607 of the Administrative Instru	of the description, c	claims and/or drawings which have ions made before this Authority	
		to the following item	ns:	
	nd corresponding pages relating	to the following near		
I X Basis of the report				
II Priority III Non-establishment of	opinion with regard to novelty, i	nventive step and in	dustrial applicability	
IV Lack of unity of inver		•		
V Reasoned statement u	nder Article 35(2) with regard to	novelty, inventive st	tep or industrial applicability;	
citations and explanati	ons supporting such statement			
VI Certain documents cit	ed			
VII Certain defects in the	international application			
VIII Certain observations	on the international application			
Date of submission of the demand	Dat	e of completion of th	nis report	
21/11/1996			1 0. 09. 97	
Name and mailing address of the IPEA/	Aut	horized officer	0 50	
European Patent Office	1	B. Heimann-Pohl		
D-80298 Munich Tel. (+49-89) 2399-0, Tx: 52	3656 epmu d	B. Heir	Haitiff Oil	
Fax: (+49-89) 2399-4465		phone No.	87.13	

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

I. B	asis of the report	
0	his report has been drawn up on the basis of (Replace ffice in response to an invitation under Article 14 a ot annexed to the report since they do not contain am	ment sheets which have been furnished to the receiving re referred to in this report as "originally filed" and are mendments.):
	[] the international application as originally fil	ed.
	pages	, as originally filed,, filed with the demand,, filed with the letter of,, filed with the letter of,
	Nos	, as originally filed,, as amended under Article 19,, filed with the demand,, filed with the letter of,, filed with the letter of,
	sheets/fig	, as originally filed,, filed with the demand,, filed with the letter of,, filed with the letter of,
	The amendments have resulted in the cancellation of: [] the description, pages	.
	considered to go beyond the disclosure as filed Additional observations, if necessary:	

Reasoned statement under Article 35(2) with regard to novelty, inventive step and ind g such statement	ustrial applicability;
STATEMENT		
Novelty (N)	Claims 2, 3, 10-13	YES
	Claims 1, 4-9	NO
Inventive Step (IS)	Claims	YES
•	Claims 2, 3, 10-13	NO
Industrial Applicability (IA)	Claims 1-13	YES
•	Claims	NO

2. CITATIONS AND EXPLANATIONS

- The present application relates to alkaline liquefying alpha-amylase activity, a DNA molecule encoding said protein, its cloning, hosts and a method for producing said protein.
- 2). D1 (WO,A,94 26881) discloses liquefying alpha-amylase from Bacillus sp. KSM-AP1378 (page 5). The protein was not sequenced and the correponding DNA not cloned.

D2 (Biochemical and Biophysical Research Communications, vol.151, no.1, 1988, pages 25-31) discloses the cloning and sequence of an alkaline liquefying alpha-amylase from Bacillus sp. 707. The sequence of said amylase appears to be different from SEQ ID NO:1 or SEQ ID NO:2 of the present application.

D3 (Journal of Biochemitry, vol.98, no.5, 1985, pages 1147-1156) describes the cloning of an alkaline liquefying alpha-amylase from B. licheniformis. The sequence of

said enzyme is also given.

3). Claim 9 is not novel with regard to D1, because D1, to which the present application refers to on page 3, discloses the same protein as the one which is claimed in claim 9.

Claim 1 is not restricted to a specific sequence therefore the subject matter of claim 1 includes the sequences disclosed in D2 and D3.

Also claims 4-9 embrace the subject matter of D2 and D3.

Therefore the subject matter of claims 1, 4-9 is not novel (Art. 33 (2) PCT).

The subject matter of claims 2, 3 and 10-13 appears to be novel with regard to D1-D3.

4). Once a protein is known in the art, as it is the case for the protein of claim 9 with regard to D1, it does not require inventive skill to sequence said protein. From the amino acid sequence the nucleotide sequence can be deduced and the gene can be isolated and cloned without inventive skill.

As a consequence claims 2, 3, 10-13 lack an inventive step (Art. 33 (3) PCT).

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The internal denominations of the plasmid in claims 12 and 13 are meaningless to the skilled person, therefore these claims are unclear (Art. 6 PCT).

ATT/5796/0/64,

Claims

- 1. A DNA molecule encoding alkaline liquefying α -amylase activity.
- 2. A DNA molecule as defined in Claim 1, which encodes the amino acid sequence described in Sequence No. 1 or a functional fragment thereof.
- 3. A DNA molecule encoding a protein exhibiting alkaline liquefying α -amylase activity and possessing an amino acid sequence described in Sequence No. 1 in which one or more amino acids are substituted, added, deleted, inverted, or inserted.
- 4. A DNA molecule as defined in any one of Claims 1 through 3, further comprising a nucleotide sequence for regulating expression of a gene.
- 5. A recombinant DNA containing the DNA molecule of any one of Claims 1 through 4.
- 6. A transformed microorganism harboring the recombinant DNA of Claim 5.
- 7. A method for producing alkaline liquefying α -amylase, comprising culturing the transformed microorganism of Claim 6 and isolating the alkaline liquefying α -amylase produced by the microorganism.
- 8. A DNA molecule which hybridizes to a DNA sequence which is complementary to the nucleic acid sequence of SEQ ID No. 2.
 - 9. A protein encoded by the DNA molecule of Claims 1 through 4.
- 10. A DNA molecule which hybridizes to a DNA sequence which is complementary to the nucleic acid sequence of SEQ ID No. 2, wherein said DNA molecule encodes a protein having alkaline

liquefying α -amylase activity.

- 11. A protein encoded by the DNA molecule of Claim 10.
- 12. The recombinant DNA plasmid pAML100.
- 13. The recombinant E. coli strain HB101(pAML100).

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notificat Preliminary	tion of Transmittal of International Examination Report (Form PCT/IPEA/416)
FP-KS-0434 International application No.	International filing date (de		Priority date (day/month/year)
			14/06/1995
PCT/ JP 96/ 01641 International Patent Classification (IPC) or	14/06/1996 national classification and IF	<u> </u>	1470071333
Thermadonal Fatent Classification (11 c) of	C12N15/56		
Applicant	0121113/30		
KAO CORPORATION et al.			
 This international preliminary example and is transmitted to the This REPORT consists of a total 	e applicant according to Artic	cle 36.	
This report is also accompan	nied by ANNEXES, i.e., she asis for this report and/or she 607 of the Administrative Ins	ets of the descripti	on, claims and/or drawings which have ifications made before this Authority
3. This report contains indications ar		ng to the following	items:
[X] Basis of the report			
II Priority			
<u></u>	opinion with regard to novelt	v. inventive step ar	nd industrial applicability
IV Lack of unity of invent		,	
V Reasoned statement un	ider Article 35(2) with regard	to novelty, invent	ive step or industrial applicability;
citations and explanation	ons supporting such statemen	Į.	
VI Certain documents cite	:d		
VII Certain defects in the i	nternational application		
VIII 🛛 Certain observations o	n the international application	n	
Date of submission of the demand	Ir	ate of completion	of this report
21/11/1996			1 0. 09. 97
Name and mailing address of the IPEA/	A	uthorized officer	0 20
European Patent Office	1	B. Hair	leimann-Pohl
D-80298 Munich Tel. (+ 49-89) 2399-0, Tx: 523	656 epmu d	B. H	leimann-Poni
Fax: (+49-89) 2399-4465		elephone No.	87.13
Form PCT TPEA:409 (cover sheet) (Januar	v 1994) (29/01/	/1997)	

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

I. Basis of the report	
 This report has been drawn up on the basis of (Replacement sheets Office in response to an invitation under Article 14 are referred not annexed to the report since they do not contain amendments.) 	d to in this report as "originally filed" and are
[] the international application as originally filed.	
[x] the description, pages 1-26	, as originally filed,, filed with the demand,, filed with the letter of,, filed with the letter of,
[x] the claims, Nos	_, as amended under Article 19, _, filed with the demand, _, filed with the letter of,
$\{\mathbf{x}\}$ the drawings, sheets/fig $1/4-4/4$ sheets/figsheets/figsheets/fig	
2. The amendments have resulted in the cancellation of: [] the description, pages	·
3. [] This report has been established as if (some of) the amendm considered to go beyond the disclosure as filed (Rule 70.2	
4. Additional observations, if necessary:	

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Reasoned statement under Article 35(2) with regard to novelty, inventive step and ing such statement	dustrial applicability;
STATEMENT		
Novelty (N)	Claims 2, 3, 10-13	
•	Claims 1, 4-9	NO
Inventive Step (IS)	Claims	YES
·	Claims 2, 3, 10-13	NO
Industrial Applicability (IA)	Claims 1-13	YES
•	Claims	NO

2. CITATIONS AND EXPLANATIONS

- 1). The present application relates to alkaline liquefying alpha-amylase activity, a DNA molecule encoding said protein, its cloning, hosts and a method for producing said protein.
- 2). D1 (WO,A,94 26881) discloses liquefying alpha-amylase from Bacillus sp. KSM-AP1378 (page 5). The protein was not sequenced and the correponding DNA not cloned.

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Claim 1 is not restricted to a specific sequence therefore the subject matter of claim 1 includes the sequences disclosed in D2 and D3.

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The subject matter of claims 2, 3 and 10-13 appears to be novel with regard to D1-D3.

4). Once a protein is known in the art, as it is the case for the protein of claim 9 with regard to D1, it does not require inventive skill to sequence said protein. From the amino acid sequence the nucleotide sequence can be deduced and the gene can be isolated and cloned without inventive skill.

As a consequence claims 2, 3, 10-13 lack an inventive step (Art. 33 (3) PCT).

Intern. application No.
PCT/JP69/01641

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The internal denominations of the plasmid in claims 12 and 13 are meaningless to the skilled person, therefore these claims are unclear (Art. 6 PCT).

- 1. A DNA molecule encoding alkaline liquefying α -amylase activity.
- 2. A DNA molecule as defined in Claim 1, which encodes the amino acid sequence described in Sequence No. 1 or a functional fragment thereof.
- 3. A DNA molecule encoding a protein exhibiting alkaline liquefying α -amylase activity and possessing an amino acid sequence described in Sequence No. 1 in which one or more amino acids are substituted, added, deleted, inverted, or inserted.
- 4. A DNA molefule as defined in any one of Claims 1 through 3, further comprising a nucleotide sequence for regulating expression of a gene.
- 5. A recombinant DNA containing the DNA molecule of any one of Claims 1 through 4.
- 6. A transformed microorganism harboring the recombinant DNA of Claim 5.
- 7. A method for producing alkaline liquefying $\alpha\text{-amylase},$ comprising culturing the transformed microorganism of Claim 6 and isolating the alkaline liquefying $\alpha\text{-amylase}$ produced by the microorganism.
- 8. A DNA molecule which hybridizes to a DNA sequence which is complementary to the nucleic acid sequence of SEQ ID No. 2.
 - 9. A protein encoded by the DNA molecule of Claim 9.
- 10. A DNA molecule which hybridizes to a DNA sequence which is complementary to the nucleic acid sequence of SEQ ID No. 2, wherein said DNA molecule encodes a protein having alkaline

liquefying α -amylase activity.

- 11. A protein encoded by the DNA molecule of Claim 11.
- 12. The recombinant DNA plasmid AML100.
- 13. The recombinant E. coli strain HB101(pAML100).

Abstract

The present invention provides a DNA/fragment encoding alkaline liquefying α -amylase, recombinant DNA containing the DNA fragment, a transformed microorganism harboring the recombinant DNA, as well as a method for producing alkaline liquefying α -amylase using the transformant. The method of the present invention enables mass production of alkaline liquefying α -amylase useful as a detergent component.

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特許手続上の改生物の評託の国際的承認 に関するブダベスト条約

下記国際高託当局によって規則7.1に従い 発行される

原寄託についての受託証

氏名 (名称)

章 啓

page.

DEPOSIT

PATENT PROCEDURE

谷託者

おて名 白 321-34

栃木県芳賀郡市員町大字赤羽2606

殿

1. 放生物の表示

(寄託者が付した識別のための表示)

Bacillus sp. KSM-AP1378

(安託番号)

放工研系容第

RUDAPEST TREATY ON THE INTERNATION NAL RECOGNITION OF THE DEPOSIT OF MICROORCANISMS FOR THE PURPOSES OF

RECEIPT IN THE CASE OF AN ORIGINAL

issued pursuant to Rule 7, I by the INTERNATIONAL DEPOSITARY AUTHORITY

identified at the bottom of this

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(FERM BP- 3048

Ⅱ、科学的性質及び分類学上の位置

「協の微生物には、次の事別を記載した文書が添付されていた。

区 科学的性質

図 分類学上の位置

Ⅲ. 受領及び受託

本国際電託当局は、平成 元年 7月24日(原常託日)に受領した「田の微生物を受託する。

呼成 元年 7月2 4日に容託された版工研図寄第 P- 10886 号より移着)

IV. 国際寄託当局 ·

通商産業省工業技術院設生物工業技術研究所

名 称:

Agency

THE RESERVENT INSCITUTE

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. DIRECTOR CENERAL.

あて名: 日本因茨版県つくば市東し『目し語3号(単圧銀号305) l-3. Nigaahi l chome Tzukuha-zhi [baraki-kun

303. JAPAN

平成 2年(1990) 3月 8日

PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING THE FILING OF AMENDMENTS OF THE CLAIMS

(PCT Administrative Instructions, Section 417)

From the INTERNATIONAL BUREAU

| To

ARUGA, Mitsuyuki
Kyodo Building
3-6, Nihonbashiningyocho 1-chome
Chuo-ku
Tokyo 103

Date of mailing (day/month/year)

13 December 1996 (13.12.96)

Applicant's or agent's file reference

FP-KS-0434

International application No.

PCT/JP96/01641

IMPORTANT NOTIFICATION

International filing date

(day/month/year)

JAPON

14 June 1996 (14.06.96)

Applicant

KAO CORPORATION et al

1. The applicant is hereby notified that amendments to the claims under Article 19 were received by the International Bureau on:

11 December 1996 (11.12.96)

2. This date is within the time limit under Rule 46.1.

Consequently, the international publication of the international application will contain the amended claims according to Rule 48.2(f), (h) and (i).

3. The applicant is reminded that the international application (description, claims and drawings) may be amended during the international preliminary examination under Chapter II, according to Article 34, and in any case, before each of the designated Offices, according to Article 28 and Rule 52, or before each of the elected Offices, according to Article 41 and Rule 78.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorised officer

K. Takeda

Telephone No.: (41-22) 730.91.11





Claims

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- 1. A DNA molecule encoding alkaline liquefying α -amylase activity.
- 2. A DNA molecule as defined in Claim 1, which encodes the amino acid sequence described in Sequence No. 1 or a functional fragment thereof.
- 3. A DNA molecule encoding a protein exhibiting alkaline liquefying α -amylase activity and possessing an amino acid sequence described in Sequence No. 1 in which one or more amino acids are substituted, added, deleted, inverted, or inserted.
- 4. A DNA molecule as defined in any one of Claims 1 through 3, further comprising a nucleotide sequence for regulating expression of a gene.
- 5. A recombinant DNA containing the DNA molecule of any one of Claims 1 through 4.
- 6. A transformed microorganism harboring the recombinant DNA of Claim 5.
- 7. A method for producing alkaline liquefying α -amylase, comprising culturing the transformed microorganism of Claim 6 and isolating the alkaline liquefying α -amylase produced by the microorganism.
- 8. A DNA molecule which hybridizes to a DNA sequence which is complementary to the nucleic acid sequence of SEQ ID No. 2.
 - 9. A protein encoded by the DNA molecule of Claims 1 through 4.
- 10. A DNA molecule which hybridizes to a DNA sequence which is complementary to the nucleic acid sequence of SEQ ID No. 2, wherein said DNA molecule encodes a protein having alkaline

liquefying α -amylase activity.

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- 11. A protein encoded by the DNA molecule of Claim 10.
- 12. The recombinant DNA plasmid pAML100.
- 13. The recombinant E. coli strain HB101(pAML100).

PCT

REQUEST

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PCT
24.06.96
International Application"

Applicant's or agent's file reference [If desired] (12 characters maximum) Box No. I TITLE OF INVENTION GENE ENCODING ALKALINE LIQUEFYING ALPHA-AMYLASE Box No. II APPLICANT Name and address: (Family name followed by given rame: for a legal entity, full official designation. The address must include postal code and name of country) Kao Corporation 14-10, Nihonbashikayabacho 1-chome, Chuo-ku, Tokyo 103 Japan State (i.e. country) of residence: Japan This person is applicant all designated as a land designated of America only in the Supplemental Box No. II FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) Name and address: (Family name followed by given rame: for a legal entity, full official of the Supplemental Box Supplemental Box No. II FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) Name and address: (Family name followed by given rame: for a legal entity, full official of the Supplemental Box Supplemental Supplemental Box Supplemental Supplemental Box Supplemental	The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.	Name of receiving Office and "PCT International Application"
Box No. I TITLE OF INVENTION GENE ENCODING ALKALINE LIQUEFYING ALPHA-AMYLASE Box No. II APPLICANT Name and address: (Family name followed by given name: for a legal entity, full official designation. The address miss include postal code and name of country.) Kao Corporation 14-10, Nihonbashikayabacho 1-chome, Chuo-ku, Tokyo 103 Japan State (i.e. country) of residence: Japan This person is applicant of the burnied States except of America only desagration. The address must include postal code and name of country.) Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) HATADA Yuji c/o Kao Corporation, Research Laboratories, 2606, Akabane, Ichikai-machi, Haga-gun, Tochigi 321-34 Japan This person is applicant and inventor with the first proposes of: State (i.e. country) of nationality: Japan This person is applicant and inventor of America only (lifthis check-box is marked. do not fill in below) State (i.e. country) of nationality: Japan This person is applicant and inventor of America only (lifthis check-box is marked. do not fill in below) State (i.e. country) of nationality: Japan This person is applicant and inventor of America only (lifthis check-box is marked. do not fill in below) State (i.e. country) of nationality: Japan This person is applicant and inventor of America only (lifthis check-box is marked. do not fill in below) State (i.e. country) of nationality: State (i.e. country) of residence: Japan This person is applicant and inventor of America only (lifthis check-box is marked. do not fill in below) State (i.e. country) of nationality: State (i.e. country) of residence: Japan This person is applicant and inventor of America only (lifthis check-box is marked. do not fill in below) State (i.e. country) of nationality: Japan This person is applicant and inventor of America only (lifthis check-box is marke		Applicant's or agent's file reference
GENE ENCODING ALKALINE LIQUEFYING ALPHA-AMYLASE Box No. II APPLICANT Name and address: (Family name followed by given name: for a legal entity, full afficial distinguistion. The address mist include postal code and name of country) Kao Corporation 14-10, Nihonbashikayabacho 1-chome, Chuo-ku, Tokyo 103 Japan State (i.e. country) of residence: Japan This person is applicant for the purposes of: Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) Name and address: (Family name followed by given name: for a legal entity, full afficial designation. The address must include fortal code and name of country) C/O Kao Corporation, Research Laboratories, 2606, Akabane, 1chikai-machi, Haga-gun, State (i.e. country) of residence: Japan This person is applicant only C/O Kao Corporation, Research Laboratories, 2606, Akabane, 1chikai-machi, Haga-gun, State (i.e. country) of residence: Japan This person is applicant only State (i.e. country) of residence: Japan This person is applicant only C/O Kao Corporation, Research Laboratories, 2606, Akabane, 1chikai-machi, Haga-gun, inventor only ((Vinis check-box dumerised do not fill in beliaw) State (i.e. country) of nationality: Japan State (i.e. country) of residence: Japan This person is applicant and inventor C/O Kao Corporation, Research Laboratories, 2606, Akabane, 1chikai-machi, Haga-gun, inventor only ((Vinis check-box dumerised)) State (i.e. country) of nationality: Japan State (i.e. country) of residence: Japan This person is applicant and inventor of the purposes of: States: (The person identified below is herebythas been appointed to act on behalf of the purposes of: States: (The person identified below is herebythas been appointed to act on behalf of the applicant(s) before the competent International Authorities as: Name and address: (Family name followed by given name: for a legal entity, full afficial designation the address that the laberation and the states of the supplemental Box of the applicant of the designation and the supplem	Box No. I TITLE OF INVENTION	15 - con cop (12 chas aciers maximum)
Name and address: (Family name followed by given name: for a legal entity, full official designation. The address mist include postal code and name of country.) Kao Corporation 14-10, Nihonbashikayabacho 1-chome, Chuo-ku, Tokyo 103 Japan State (i.e. country) of nationality: Japan This person is applicant for the purposes of: Bax No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) Name and address: (Family name followed by given name: for a legal entity, full official designation and inventor only (If this check-box is marked, do not fill in below) State (i.e. country) of nationality: Japan This person is applicant for the purposes of: Bax No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) Name and address: (Family name followed by given name: for a legal entity, full official designation. The address mist include postal code and name of country.) C/O Kao Corporation, Research Laboratories, 2606, Akabane, Ichikai-machi, Haga-gun, Tochigi 321-34 Japan State (i.e. country) of nationality: Japan State (i.e. country) of nationality: Japan This person is applicant for the purposes of: This person is applicant and inventor only (If his check-box is marked, do not fill in below) is marked. do not fill in below) State (i.e. country) of nationality: Japan State (i.e. country) of nationality: Japan This person is applicant and inventor only (If his check-box is marked. do not fill in below) This person is applicant for the purposes of: The purposes of: State (i.e. country) of residence: Japan This person is applicant and inventor only (If his check-box is marked. do not fill in below) This person is applicant for the purposes of: The purposes of: This person is applicant and inventor only (If his check-box is marked. do not fill in below) This person is applicant for the purposes of: This person is applicant only the state of America only inventor only (If his check-box is marked. do not fill in below) This person is applicant for the purposes of: This person is applicant for the pu	ł	YING ALPHA-AMYLASE
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Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) HATADA Yuji C/O Kao Corporation, Research Laboratories, 2606, Akabane, Ichikai-machi, Haga-gun, inventor only (If this check-box is marked, do not fill in below.) State (i.e. country) of nationality: Japan State (i.e. country) of residence: Japan This person is applicant of states and/or (further) inventors are indicated on a continuation sheet. Box No. IV AGENTOR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) 6870 Patent Attorney, ARUGA Mitsuyuki 7756 Patent Attorney, NAKAJIMA Toshio 10131 Patent Attorney, NAKAJIMA Toshio 1-chome, Chuo-ku, Tokyo 103 Japan Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to		
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Tochigi 321-34 Japan State (i.e. country) of nationality: Japan State (i.e. country) of nationality: Japan This person is applicant for the purposes of: States and/or (further) inventors are indicated on a continuation sheet. Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) 6870 Patent Attorney, ARUGA Mitsuyuki 7756 Patent Attorney, ARUGA Mitsuyuki 9673 Patent Attorney, NAKAJIMA Toshio 10131 Patent Attorney, NAKAJIMA Toshio 10131 Patent Attorney, MATOBA Hiromi all of Kyodo Bldg., 3-6, Nihonbashiningyocho Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to	•	applicant only
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orm PCT/RO/101 (first sheet) (5 July 1994), service L. 1995)	Mark this check-box where no agent or common representa-	sino ia/han h
	orm PCT/RO/101 (first sheet) (5 July 1994; reprint July 1995)	See Notes to the request form

Sheet No. Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS If none of the following sub-boxes is used, this sheet is not to be included in the request. (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) This person is: OZAKI Katsuya applicant only c/o Kao Corporation, Research Laboratories, applicant and inventor 2606, Akabane, Ichikai-machi, Haga-gun, Tochigi 321-34 Japan inventor only (If this check-box is marked, do not fill in below.) State (i.e. country) of residence: Japan Japan all designated all designated States except the United States the States indicated in Х the United States of America the Supplemental Box of America only This person is: applicant only applicant and inventor inventor only (If this check-box is marked, do not fill in below.) State (i.e. country) of residence: Japan Japan all designated all designated States except the United States the States indicated in States the United States of America of America only the Supplemental Box This person is: applicant only

State (i.e. country) of nationality: This person is applicant for the purposes of: Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) ARA Katsutoshi c/o Kao Corporation, Research Laboratories, 2606, Akabane, Ichikai-machi, Haga-gun, Tochigi 321-34 Japan State (i.e. country) of nationality: This person is applicant for the purposes of: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.) Name and address: KAWAI Shuji c/o Kao Corporation, Research Laboratories, applicant and inventor 2606, Akabane, Ichikai-machi, Haga-gun, Tochigi 321-34 Japan inventor only (If this check-box is marked, do not fill in below.) State (i.e. country) of nationality: State (i.e. country) of residence: Japan Japan This person is applicant all designated all designated States except the United States the States indicated in for the purposes of: States the United States of America of America only the Supplemental Box (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) Name and address: This person is: ITO Susumu applicant only c/o Kao Corporation, Research Laboratories, applicant and inventor 2606, Akabane, Ichikai-machi, Haga-gun, Tochigi 321-34 Japan inventor only (If this check-box is marked, do not fill in below.) State (i.e. country) of nationality: State (i.e. country) of residence: Japan Japan This person is applicant all designated all designated States except the United States of America the States indicated in the United States for the purposes of: of America only the Supplemental Box

Form PCT/RO/101 (continuation sheet) (July 1993; reprint July 1995)

Further applicants and/or (further) inventors are indicated on another continuation sheet.

Name and address:

Box	No.V	DESIGNATION OF STATES			·		
The	The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):						
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	AP	ARIPO Patent: KE Kenya, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda and any other State which is a Contracting State of the Harare Protocol and of the PCT					
X	EP	ES Spain, FR France, GB United Kingdom, G	R Gr	eece,	tzerland and Liechtenstein, DE Germany, DK Denmark, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, te which is a Contracting State of the European Patent		
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In ad	dition	to the designations made above, the applicant also	makes	unde	Rule 4.9(b) all designations which would be permitted		
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before	the e	xpiration of 15 months from the priority date is to be r	egardo	ed as v	vithdrawn by the applicant at the expiration of that time		
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Box No. VI PRIORITY C	LAIM	Fu	ther priority claim	s are indicated in t	the Supplemental Box
The priority of the following ex	arlier application	(s) is hereby claimed	1:		
Country (in which, or for which, the application was filed)	Filir (day/m	ng Date conth/year)	Applicat	ion No.	Office of filing (only for regional or international application)
item (1) Japan	14.00	6.95	Patent Appl: 7-1472		
item (2)	·				
item (3)					
Mark the following check-box if the application is the receiving Office The receiving Office is h Bureau a certified copy of	(a fee may be requ	ired):			poses of the present international
Box No. VII INTERNATIO	NAL SEARCH	ING AUTHORITY	•		
Choice of International Search are competent to carry out the international Search Fill in where a search out or requested and the Authority is such search or request either by recountry (or regional Office):	national search, ind wch (international, s now requested to d ference to the relev	dicate the Authority ch international-type or t base the international	osen; the two-letter co other) by the Internat: search, to the extent n	nde may be used): 1 ional Searching Aut ossible, on the result	hority has already been carried ts of that earlier search Identify
Box No. VIII CHECK LIST	•				
2. description : 25 3. claims : 2 4. abstract : 1 5. drawings : 4 Total : 36 Figure No of the	sheets sheets sheets sheets sheets sheets sheets drawings (if any OF APPLICAN) The of the person sign	1. X separate power of copy of power of power of statemen lack of s 4. priority of identified as item(s) should accompany T OR AGENT sing and the capacity in the company in the capacity in the copy in the copy in the copy in the capacity in the capaci	signed attorney general attorney t explaining gnature document(s) t in Box No. VI the abstract when which the person signs (5. X fee calc 6. X separat deposit 7. nucleot sequence 8. other (stransfine) Document is published.	
Date of actual receipt of the international application: Corrected date of actual receitimely received papers or drathe purported international approach.	ipt due to later bu	ut	ffice use only		2. Drawings:
4. Date of timely receipt of the corrections under PCT Article	required le 11(2):				not received:
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PCI	For receiving Office use only
FEE CALCULATION SHEET	
Annex to the Request	International application No.
Applicant's or agent's file reference FP-KS-0434	Date stamp of the receiving Office
Applicant Kao Corporation	
Nuo Colpolution	
CALCULATION OF PRESCRIBED FEES	
1. TRANSMITTAL FEE	18,000 T
2. SEARCH FEE	169,000 S
International search to be carried out by	
(If two or more International Searching Authorities are competent in rela application, indicate the name of the Authority which is chosen to carry out	the international the international search.)
3. INTERNATIONAL FEE	
Basic Fee The international application contains 36 sheets.	
first 30 sheets	00 b ₁
$\frac{6}{}$ x $\frac{1,300}{}$ = $\frac{7,8}{}$	
remaining sheets additional amount	
Add amounts entered at b ₁ and b ₂ and enter total at B	75,200 B
Designation Fee	
$\frac{3}{2}$ x $\frac{16,400}{2}$ =	49,200 D
number of designations amount of designation fee	
(If that total exceeds the figure which corresponds to the amount of the designation fee multiplied by ten, enter the latter figure in box D.)	
Add amounts entered at B and D and enter total at I	124,400 [
4. FEE FOR PRIORITY DOCUMENT	P P
5. TOTAL FEES PAYABLE	
Add amounts entered at T, S, I and P, and enter total in the TOTAL box	311,400
and office form in the 1017th box	TOTAL
The designation fee is not paid at this time.	
MODE OF PAYMENT	
authorization to charge deposit account (see below) bank draft	coupons
cheque cash	other (specify):
postal money order revenue stamps	Since (epassy).
DEPOSIT ACCOUNT AUTHORIZATION (this mode of payment mo	
The RO/ is hereby authorized to charge the total fees in	
is hereby authorized to charge any deficiency deposit account.	y or credit any overpayment in the total fees indicated above to my
is hereby authorized to charge the fee for prep Bureau of WIPO to my deposit account.	paration and transmittal of the priority document to the International
Bureau of wife to my deposit account.	
Deposit Account Number Date (day/month/year)	Signature
Deposit Account Number Date (day/month/year)	Signature



PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER see Notification (Form PCT/ISA	of Transmittal of International Search Report /220) as well as, where applicable, item 5 below.
FP-KS-0434	International filing date(day/month/year)	(Earliest) Priority Date (day/month/year)
International application No.		
PCT/JP 96/01641	14/06/1996	14/06/1995
Applicant		
KAO CORPORATION et al.		
This International Search Report has according to Article 18. A copy is being the copy in the copy is being the copy in the copy is being the copy in	been prepared by this International Searching Aung transmitted to the International Bureau.	uthority and is transmitted to the applicant
This International Search Report con It is also accompanied by a	sists of a total of 3 sheets. copy of each prior art document cited in this rep	ort
1. Certain claims were found u	insearchable (see Box I).	,
2. Unity of invention is lacking	(see Box II).	
3. X The international application international search was ca	on contains disclosure of a nucleotide and/or amin rried out on the basis of the sequence listing	o acid sequence listing and the
	filed with the international application.	
$\overline{\mathbf{x}}$	furnished by the applicant separately from the ir	nternational application,
	but not accompanied by a statement to matter going beyond the disclosure in t	the effect that it did not include he international application as filed.
	Transcribed by this Authority	
4. With regard to the title , X	the text is approved as submitted by the applica	
	the text has been established by this Authority t	o read as follows:
5. With regard to the abstract,	the text is approved as submitted by the applica	unt.
	the text has been established, according to Rule Box III. The applicant may, within one month Search Report, submit comments to this Author	from the date of maning of and more
6. The figure of the drawings to be		N Cabo Gaussa
Figure No	as suggested by the applicant.	None of the figures.
	because the applicant failed to suggest a figure.	
	because this figure better characterizes the inve	ntion.
1		

International Application No PCT/JP 96/01641

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/56 C12N9/28

C12N1/21

C12N15/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Х	WO,A,94 26881 (KAO CORP; ARA KATSUTOSHI (JP); SAEKI KATSUHISA (JP); IGARASHI KAZU) 24 November 1994 cited in the application see the whole document & EP,A,0 670 367 (KAO CORPORATION)	1-13
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 151, no. 1, 29 February 1988, pages 25-31, XP000605386 TSUKAMOTO A. ET AL.: "Nucleotide sequence of the maltohexaose-producing amylase gene from alkalophilic Bacillus sp. #707 and structural similarity to liquefying type	1,3-11
A	alpha-amylases." see the whole document/	2,12,13

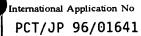
* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but
"A" document defining the general state of the art which is not considered to be of particular relevance	cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to
"L" document which may throw doubts on priority claim(s) or	involve an inventive step when the document is taken alone
which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the
"O" document referring to an oral disclosure, use, exhibition or other means	document is combined with one or more other such docu- ments, such combination being obvious to a person skilled
P document published prior to the international filing date but later than the priority date claimed	in the art. "&" document member of the same patent family
Date of the actual completion of the international search	Date of mailing of the international search report
6 November 1996	1 5. 11. 96
Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Mandl, B

Form PCT/ISA/210 (second sheet) (July 1992)

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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.



Category *	citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOCHEMISTRY, vol. 98, no. 5, 1985, pages 1147-1156, XP002017641 YUUKI T ET AL.: "Complete nucleotide sequence of a gene coding for heat- and pH-stable alpha-amylase of Bacillus licheniformis: Comparison of the amino acid sequences of three bacterial liquefying alpha-amylases deduced from the DNA sequences."	1,3-11
Α	see the whole document	2,12,13
A	EP,A,O 410 498 (GIST BROCADES NV ;PLANT GENETIC SYSTEMS NV (BE)) 30 January 1991 see the whole document	3
P,X	WO,A,95 26397 (NOVONORDISK AS ;OUTTRUP HELLE (DK); BISGAARD FRANTZEN HENRIK (DK);) 5 October 1995 see the whole document	1,3-11
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ERNATIONAL SEARCH REPORT

hitomation on patent family members

International Application No PCT/JP 96/01641

Patent family Publication Publication Patent document date cited in search report member(s) 11-10-95 WO-A-9426881 24-11-94 CN-A-1110058 EP-A-0670367 06-09-95 24-06-93 30-01-91 AU-B-638263 EP-A-0410498 5953890 17-01-91 AU-A-CA-A-2030554 30-12-90 CN-A-1050220 27-03-91 10-01-91 WO-A-9100353 13-02-92 JP-T-4500756 5364782 15-11-94 US-A-17-10-95 WO-A-9526397 05-10-95 AU-A-2067795 9502565 21-12-95 ZA-A-